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A review on the phytochemicals and antioxidant activity of *Polygonum glabrum L.*

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Abstract: *Free radicals and oxidants give rise to a phenomenon known as oxidative stress; this is a harmful process that can negatively affect several cellular structures, such as membranes, lipids, proteins, lipoproteins and deoxyribonucleic acid (DNA) [13–18]. Oxidative stress emerges when an imbalance exists between free radical formation and the capability of cells to clear them. Antioxidants are substances that can neutralize the free radicals by donating and electron, this neutralizing effect helps protect the body from oxidative stress. Studies have shown that Polygonum glabrum has proved to contain high concentrations of antioxidants and can possibly be implemented in preventing and treating oxidative stress related ailments. This review is an effort to study the antioxidative properties of Polygonum glabrum, and how it may help as a medicine to tackle different ailments caused by oxidative stress in the body and to shed a light into the importance of medicinal plants and to update the phytochemical data of the plant*

Keywords: Antioxidant, *polygonum glabrum*, radicals

1.1 INTRODUCTION:

Oxidation is a process defined as the loss of electrons during a reaction by a molecule, atom or ion, which may occur spontaneously or artificially. Due to various biological processes, this loss of electrons can occur in cells and tissues which may be harmful if not controlled. Biologically, certain damages on cells and tissues are caused by unstable molecules, free radicals, produced in the body as a reaction to environmental condition, certain food materials, stress and other factors. These substances are termed as oxidants and free radicals.

When an oxygen molecule splits into single atoms with unpaired electrons, they are called free radicals. When atoms are molecules gain or lose electrons due to various metabolic activities and other factors, these free radicals are released in the body.**[1,2]** Electrons like to be in pairs, so these free radicals, scavenge the body to seek out other electrons so they can become a pair**.** Free radicals are formed naturally in the human body when exercise is performed or when the body metabolize food and converts it into energy and by exposure to cigarette smoke, pollution and sunlight **[3].**

1.2 Oxidants and Free radical Production:

Superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($1O_2$) are commonly defined reactive oxygen species (ROS); they are generated as metabolic by-products by biological systems [1, 2]. When ROS production increases, they start showing harmful effects on important cellular structures like proteins, lipids, and nucleic acids [4]. A large body of evidences shows that oxidative stress can be responsible, with different degrees of importance in the onset and/or progression of several diseases (i.e. Cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases) [5]. ROS are mainly produced by mitochondria, during both physiological and pathological conditions, that is, $O_2^{\bullet-}$ can be formed by cellular respiration [6].

ROS production basically relies on enzymatic and non enzymatic reactions. Enzymatic reactions able to generate ROS are those involved in respiratory chain, prostaglandin synthesis, phagocytises, and cytochrome P_{450} system [7–17]. Even no enzymatic reactions can be responsible for free radical production, that is, when oxygen reacts with organic compounds or when cells are exposed to ionizing radiations. No enzymatic free radical production can occur as well during mitochondrial respiration [12, 13, and 16].

Free radicals are generated from both endogenous and exogenous sources. Immune cell activation, inflammation, ischemia, infection, cancer, excessive exercise, mental stress, and aging are all responsible for endogenous free radical production. Exogenous stimulants for free radical production include environmental pollutants, heavy metals (Cod, Hg, BP, Fe, and As), certain drugs (cyclosporine, tacrolimus, gentamycin, and bleomycin), chemical solvents, cooking (smoked

meat, used oil, and fat), cigarette smoke, alcohol, and radiations [12–22]. When these exogenous compounds penetrate the body, they are degraded or metabolized, and free radicals are generated as by-products.

1.3 Physiological activities of free radicals:

Free radicals interact chemically with cell components such as DNA, RNA , Lipids, proteins and take away their electrons to become stabilized and subsequently causes damage to the DNA, RNA, Lipids and Proteins. This also destabilizes the cell component molecules which then seek and take away electron from another molecule, therefore triggering a large chain of free radical reactions **[4, 5].**

When maintained at low or moderate concentrations, free radicals play several beneficial roles for the organism. For example, they are needed to synthesize some cellular structures and to be used by the host defence system to fight pathogens. In fact, phagocytes synthesize and store free radicals, in order to be able to release them when invading pathogenic microbes have to be destroyed [13, 18]. Free radicals are also involved in a number of cellular signalling pathways [15–17].Free radicals play a key regulatory role in intracellular signalling cascades, in several cell types such as fibroblasts, endothelial cells, vascular smooth muscle cells, cardiac myocytes, and thyroid tissue. Another physiological activity of free radicals is the induction of a mitogenic response [15, 16]. Summarizing, free radicals, when maintained at low or moderate levels, are of crucial importance to human health but an imbalance of free radicals and antioxidants in the human body can also trigger oxidative stress leading to problems such as tissue and cell damage.

1.4 Detrimental Effects of Free radicals:

As stated before, if in excess, free radicals and oxidants give rise to a phenomenon known as oxidative stress; this is a harmful process that can negatively affect several cellular structures, such as membranes, lipids, proteins, lipoproteins and deoxyribonucleic acid (DNA) [13–18]. Oxidative stress emerges when an imbalance exists between free radical formation and the capability of cells to clear them. For instance, an excess of hydroxyl radical and peroxy nitrite can cause lipid per oxidation, thus damaging cell membranes and lipoproteins. This in turn will lead to malondialdehyde (MDA) and conjugated diene compound formation, which are known to be cytotoxic as well as mutagenic. Being a radical chain reaction, lipid per oxidation spreads very quickly affecting a large amount of lipid molecules [22]. Proteins may as well being damaged by oxidative stress, undergoing to conformational modifications that could determine a loss, or impairment, of their enzymatic activity [17, 22].

If not strictly controlled, oxidative stress can be responsible for the induction of several diseases, both chronic and degenerative, as well as speeding up body aging process and cause acute pathologies (i.e., trauma and stroke).

1.5 Oxidative stress: When the human body is unable to process and remove free radicals efficiently, Oxidative stress occurs. This can harm cells, tissues and body function [6]. Oxidation is a naturally occurring process and plays a role in the process of aging. Another source of reactive oxygen under normal conditions in humans is the leakage of activated oxygen from mitochondria during the process of oxidative phosphorylation. **[23, 24, 25]**Oxidation damage is highly dependent on the acquired defects in enzymes involved in the redox-mediated signaling pathways. Research and scientific evidence shows that oxidative stress can lead to various chronic conditions including cancer, diabetes, cardiovascular diseases etc. **[3]**. The body's immune response system can also cause oxidative stress temporarily. If oxidative stress is uncontrolled, it can also lead to acceleration of aging process and cause various ailments **[7].**Some reactive oxidative species (ROS) can act as cellular messenger in redox signaling. Hence oxidative stress can cause disturbances in normal cell signaling mechanism. The electron transport chain consumes up to 90% of total oxygen (O_2) taken up by the cells. During this process, as by products, Reactive Oxygen Species (ROS) are generated for the partial four-electron reduction of O_2 to produce water molecule, the last electron acceptor in the ATP generation process.

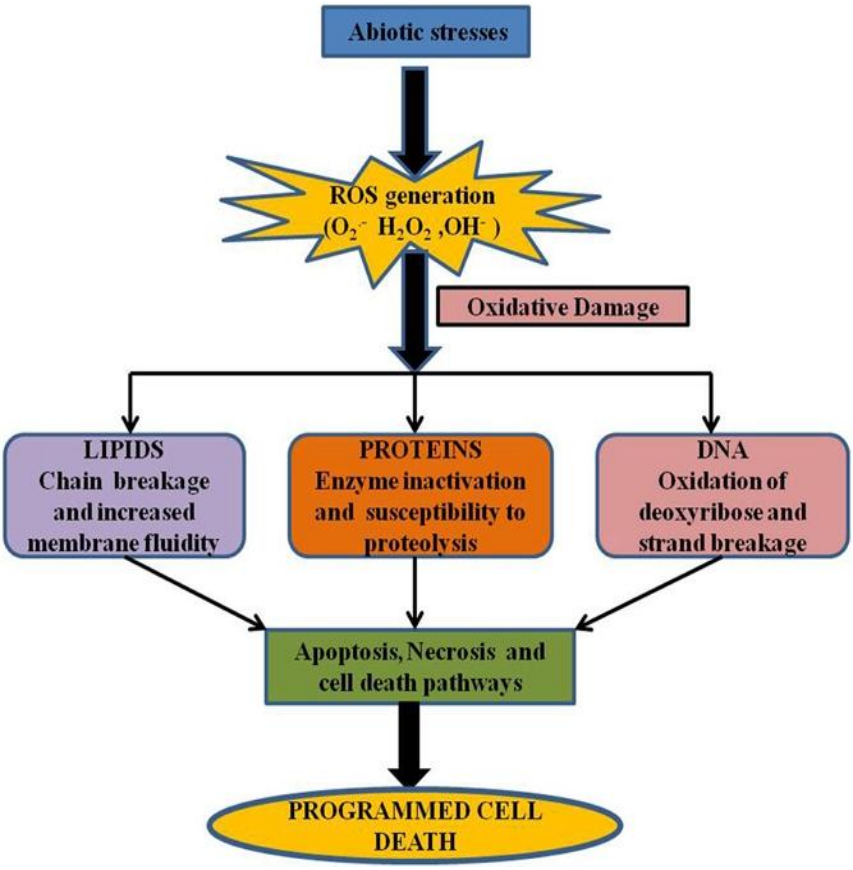

Fig 2- Reactive Oxygen Species and effects of Oxidative stress

Some conditions link to oxidative stress include cancer, Alzheimer's disease, Parkinson's disease, high blood pressure, atherosclerosis, inflammatory disorders, chronic fatigue syndrome, asthma, male infertility etc.[8,9].Reactive oxygen species can sometimes also be beneficial for the human body, as they are used by immune response system as a way to attack and destroy pathogens. The hypothesis of oxidative stress highlights the role of antioxidant defenses as an important component of the overall redox balance of the organism.

1.6 Role of antioxidants:

Antioxidants are substances that can neutralize the free radicals by donating and electron, this neutralizing effect helps protect the body from oxidative stress [10]. Examples of such antioxidants include Vitamin A, C and E. Antioxidants come from several different sources, an antioxidant produced naturally in the body is Glutathione [11]. Antioxidants can prevent or slowdown the damage caused by free radicals, unstable molecules to cells and tissues. Some antioxidants lessen or stop the formation of free radicals, and some "scavenge" to remove free radicals before they do damage, or work to repair damage once it has been done.[12].Some common antioxidants are vitamins C and E, beta carotene, and selenium; many others are phytochemicals, such as quercetin and other flavonoids; enzymes such as glutathione-transferees, peroxidase and superoxide dismutase.[13]. Antioxidants have been known to prevent various diseases caused by oxidative stress. The degrading effects of oxidants in the body can be cancelled out by antioxidants. With years of research the role of antioxidants in the treatment and prevention of various diseases was recognized. This lead to a worldwide search for plants possessing antioxidative properties.

1.7 Plant profile:

Plants believed to possess these attributes are screened to find out various pharmacological properties. Plants of the genus of species *polygonum* have been used all over the world for its medical properties. In this study, the plant species *Polygonum glabrum L* has been selected for its report of high antioxidative capabilities. *Polygonum glabrum is* a perennial plant commonly known as dense flower knotweed. It is amphibious and grows in or closes by water bodies such as rivers and the ditches. *Polygonum glabrum L* is found all over the world, regions including Africa, Pacific Islands, North South America, Bahamas, Iran, Bangladesh, Pakistan etc. it is also found to abundantly grow in East Asian countries pneumonia, colic pain and the paste is applied on cuts and wounds**.** [17] *Polygonum glabrum L* belongs to the family *polygonaceae* and Genus *polygonum* [18]. It is 34 feet high and erect glabrous with dilated nodes. Stems have an extension of 5 – 15 centimeters in length and have a reddish colour, rooting is also generally seen in the nodes. Leaves are simple alternate and stipulate with leaf apices narrowly acuminate. Leaves are lanceolate or oblong lanceolate with petiole of 8-9 millimeters in size. Flowers are generally seen in the month of June they are bi sexual and pedicillate. Flowers are white or pink with parted petals having 6-8 stamens, 2 styles and connate. [19,20,21]

Fig 4: *Polygonum glabrum L*

This review is an effort to study the antioxidative properties of Polygonum glabrum, and how it may help as a medicine to tackle different ailments caused by oxidative stress in the body and to shed a light into the importance of medicinal plants and to update the phytochemical data of the plant.

1.8 PHYTOCHEMICAL SCREENING:

The preliminary phytochemical screening for methanol extract of the plant are performed for the identification of various plant constituents namely alkaloids flavonoids, sterols, tripterpenes, Saponins and coumarins. Preliminary Screening was carried out using techniques described by *Martinez et al. 1999, Sofowora, 1993 and Harborne, 1998.*[26,27,28]

1.8.1 Detection of Alkaloids- [29]

Extracts where basified with ammonia, extracted with chloroform. Extracts were dissolved in dilute hydrochloric acid and filtered. The acid layer obtained was tested for alkaloids.

1.8.1.1 Wagner's test (Iodine in Potassium Iodide):

The acid layer was treated with few drops of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

1.8.1.2 Dragendroff's reagent (Potassium Bismuth Iodide):

The acid layer was treated with few drops of Dragendroff's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

1.8.2 Detection of flavonoids- [29]

1.8.2.1 Potassium hydroxide (5%) test-

To one ml of extract one ml of 5% potassium hydroxide was added. Formation of bright yellow colour indicates presence of flavonoids.

1.8.2.2 Aluminum chloride (5%) test

Extract was treated with 5% aluminum chloride, which showed formation of yellow colour indicating the presence of flavonoids.

1.8.3 Detection of Sterols [29]

Extracts were dissolved in chloroform, filtered and tested for sterols and triterpenes.

1.8.3.1Salkowski test

To the chloroform solution, few drops of conc. Sulphuric acid were added and allowed to stand, appearance of red colour in lower layer indicates the presence of sterols.

1.8.3.2 Liebermann-Burchard

Few drops of acetic anhydride were added and mixed well in the chloroform solution. 1 ml of concentrated sulphuric acid was added from the sides of the test tube, appearance of reddish brown ring indicates the presence of sterols.

1.8.4 Detection of triterpenes [29]

1.8.4.1 Salkowski test:

To the chloroform solution, few drops of concentrated Sulphuric acid was added, shaken and allowed to stand, appearance of golden yellow colour indicates the presence of triterpenes.

1.8.4.2 Liebermann-Burchard:

Few drops of acetic anhydride was added and mixed in chloroform solution. 1 ml of concentrated sulphuric acid was added from the sides of the test tube, appearance of deep red colour indicates the presence of tripterpenes.

1.8.5 DETECTION OF TANNINS[29]

1.8.5.1 Ferric chloride test:

A few drops of 1% neutral ferric chloride solution were added to the extract, formation of blackish blue colour indicates the presence of tannins.

1.8.5.3 Lead acetate test:

A few drops of aqueous basic lead acetate solution were added to the extract. Reddish brown bulky precipitate indicates presence of tannins.

1.8.6 Detection of saponins[29]

1.8.6.1 Foam test:

Small amount of extract was shaken with little quantity of water, if foam produced persists for 10 minutes; it indicates the presence of saponins.**[29]**

1.8.7 DETECTION OF COUMARINS- [29]

1 g of the extract was kept with water in a test tube, covered with paper soaked in NaOH then diluted and boiled. Yellow fluorescence observed after examination under ultra-violet lamp indicates presence of coumarins

TABLE 1- Yield percentage of extract of phytochemicals of each part of plant (methanol extract) [29]

Methanol extract of plant part	Yield %
Leaves	2.8
Stems	2.4
Flowers	2.2
Root barks	0.7

Table 2: Phytochemical screening of different parts of *Polygonal glabrum.*[29] *Key: _: indicates absence of the constituents, +: low concentration, ++: moderate concentration, +++: high concentration.*

Phytochemicals	Leaves	Stems	Flowers	Root	Adventitious roots
Alkaloids	+++	+++	++	++	+
Flavonoids	+++	+++	++	++	++
Sterols	++	-	++	-	++
Triterpenes	++	-	++	++	-
Tannins	+++	+++	+++	+++	+++
Saponins	+	++	+	-	-
Coumarins	+++	++	+	++	+

1.9 Antioxidant activity

1.9.1 Evaluation by DPPH assay:

Evaluation of antioxidative activities of Polygonum glabrum was carried out by DPPH (2,2- diphenyl-1-1-picryl-hydrazyl-hydrate) assay. DPPH free radical method is based on electron transfer which produces a violet solution in ethanol and is reduced in the presence of an antioxidant molecule giving a colourless ethanol solution. This method has been used widely for antioxidant activity [30]IC50 (efficient concentration) value is used for interpretation of results. The IC50 value was calculated for each of the extract and the standard. The results are shown in Table 3

TABLE 3: Radical Scavenging Activity percentage (RSA%) of *Polygonum glabrum* extracts using DPPH assay [29]

EXTRACT (Methanol)	%RSA ± SD	IC_{50} ± SD (mg/ml)
Leaves	66.3 ± 0.19	3.193 ± 0.03
Stems	61.4 ± 0.09	2.524 ± 0.01
Flowers	91.6 ± 0.03	4.134 ± 0. It01
Root-barks	81.2 ± 0.04	3.809 ± 0.06
Adventitious Root	85.0 ± 0.01	1.361 ± 0.01
Standard	91.1 ± 0.02	3.13 ± 0.02

The results show that extracts of *Polygonum glabrum* have hydrogen donor compound, scavenging DPPH free radical at varying rates. In methanol extracts tested, extract of flowers had DPPH radical scavenging activities (91.6 ± 0.03%, IC_{50} = 4.134 ± 0.01 mg/ml), adventitious-roots methanol extract (85.0±0.01%, IC_{50} = 1.361± 0.01 mg/ml) and root-bark methanol extract (81.2 ± 0.04%, IC_{50} = 3.809 ± 0.06 mg/ml). IC_{50} concentration and the antioxidant capacity have inversely proportional values, adventitious-roots methanol extract (IC_{50} = 1.361 ± 0.01mg/ml) was found to have the highest antioxidant capacity when compared with standard (3.13 ± 0.02 mg/ml)

1.9.2 Evaluation of Antioxidant activity by FRAP (Ferric reducing Antioxidant Power) assay:

FRAP(Ferric reducing Antioxidant power) assay was used to evaluate the antioxidant activity of leaf extracts of *Polygonum glabrum.* The FRAP assay depends upon the reduction of Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion at low pH. This causes formation of a coloured ferrous probe complex from a colourless probe complex. FRAP assay is used to evaluate antioxidative activities of natural products because it is simple and easy to perform and is reproducible [31]

Table 4: FRAP assay evaluation of leaf extract[32]

Extract	**FRAP □EC(mM/mg)**
Leaves	$3.88 \pm 0.02_{ab}$
Control(Ascorbic acid)*	$3.88 \pm 0.02_{ab}$

□ *Ferric reducing ability of plasma; EC: equivalent concentration; (mM/mg)- mM Fe^{2+} per mg dry weight of plant extract.*

 Positive controls.

The following data was obtained, which shows that extracts of *Polygonum glabrum L* has Ferric to Ferrous reducing capacity. In methanol leaf extracts tested, they showed FRAP assay capability. [32]

1.10 CONCLUSION:

Oxidation is a process which occurs in the body naturally or due to artificial reasons and causes detrimental effects on the human body. Due to the action of free radicals and Reactive Species of Oxygen, Nitrogen, Hydrogen etcetera in the body, which ultimately lead to oxidative stress, causing various ailments. Researchers are implementing new strategies to help prevent and treat these diseases by experimenting on the antioxidant properties of medicinal plants. Plants of the genus *Polygonum* have proved to possess high antioxidative capabilities and have proved to be a stepping stone into discovering better ways to help prevent and treat various diseases related to oxidative stress.

Studies have shown that *Polygonum glabrum* has proved to contain high concentrations of antioxidants and can possibly be implemented in preventing and treating oxidative stress related ailments. Also by way of phytochemical screening experiments research studies shows that *Polygonum glabrum* contains secondary metabolites such as alkaloids, flavonoids, sterols, tripterpenes, Saponins and coumarins which causes antioxidant effects and other beneficial medicinal effects. It is observed that *Polygonum glabrum* lives up to its claims on being a beneficial medicinal plant with its high antioxidant effects. Hence a more in-depth research should be done on such plants to tap into the benefits they can provide for the possibility of discovering potential new drugs or to act as an alternative to prescribed pharmaceutical medicine.

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